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Effects of Small Quantities of Cornstarch and Dextrose on the Oyster, Crassostrea virginica (Gmelin)

Kenneth Wayne Turgeon

College of William and Mary - Virginia Institute of Marine Science

EFFECTS OF SMALL QUANTITIES OF CORNSTARCH AND DEXTROSE ON THE OYSTER, *Crassostrea virginica* (Gmelin)

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Kenneth Wayne Turgeon

Kenneth Wayne Turgeon

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Dexter S. Haven, M.S.

Morris L. Brehmer, Ph.D.

Marvin L. Wass, Ph.D.

John J. Norcross, M.S.

Willard A. Van Engel, PH.M.

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ABSTRACT

Effects of small quantities of cornstarch and dextrose on oysters were evaluated. Parameters measured during summer, fall and spring were glycogen, tissue weight and shell size. Cornstarch had a significantly positive influence on glycogen content, tissue weight and shell size, while effect of dextrose in solution and adsorbed on montmorillonite was limited to glycogen content. Effect of cornstarch was greatest in early fall and late spring, periods when oysters would normally accumulate glycogen. At these seasons high glycogen levels produced by cornstarch were accompanied by significant increases in wet tissue weight and shell size. A correlation between glycogen content and wet tissue weight was demonstrated.

EFFECTS OF SMALL QUANTITIES OF CORNSTARCH AND DEXTROSE ON THE OYSTER, *Crassostrea virginica* (Gmelin)

INTRODUCTION

The growth and fattening of the American oyster, Crassostrea virginica (Gmelin), has long been of interest to oyster biologists (Moore and Pope, 1910; Korringa, 1952; Gillespie, Ingle and Havens, 1966). As a commercial product, the market value of oysters depends on "meat" yield per bushel of oysters. Previous authors have shown that the amount of stored glycogen in oysters is a major factor influencing tissue size and quality (Mitchell, 1917, 1918; Engle, 1950). Mitchell (1917, 1918), Hopkins, Mackin and Menzel (1953), Engle (1950, 1957), and Galtsoff (1964) have related seasonal glycogen changes to the sexual cycle of the oyster. Stored glycogen reaches its minimum level during the summer spawning period since it is rapidly used in the formation of sex products. When spawning has ended in the fall, glycogen is stored prior to winter dormancy. During dormancy the glycogen level decreases. In spring there is an increase in glycogen prior to gonadal development. As a consequence of this seasonal change in glycogen, effects of supplements added at various periods might be expected to vary, but previous authors have never adequately investigated this possibility.

Many early workers attempted, with inconclusive results, to demonstrate the nutritional value of naturally occurring substances such as algae, protozoans and detritus /Martin, 1923, 1927a, 1927b, 1928; Mitchell, 1917; Gavard, 1927 (in Haven, 1965); Nelson, 1947/.

Other investigators, with equally conflicting results, have applied fertilizers to oyster beds to increase the nutrient level and thus increase the growth rate and condition of the oysters /Lambert, 1950, and Rochford, 1951 (both in Korringa, 1952)/.

Mitchell (1917) was the first to study effects of carbohydrates of known composition and concentration on oysters. He showed that oysters held in standing seawater containing 0.25% glucose for two to five days had more glycogen than controls. This study is unrealistic since 0.25% (2.5 g/l) is a very high concentration which is never encountered in nature. Also, studies at the Virginia Institute of Marine Science showed that this concentration not only failed to cause an increase in tissue size but was toxic (Haven, personal communication). Yonge (1928) showed active removal of dextrose from the water by Ostrea edulis. However, he did not measure possible increases in glycogen levels, and mechanism of uptake was not clear. Nelson (1934) was the first to study effects of particulate carbohydrates on oysters. While all results were not clear, only cornstarch was successful. Collier et al. (1953) showed that the pumping rate of C. virginica increased as the level of a dissolved carbohydrate-like substance in the water increased. They also showed that an oyster was capable of removing up to 50 mg/l per hour. Gillespie, Ingle and Havens (1964) showed that oysters receiving dextrose at 30 mg/l lived 68.2 days longer than starved oysters. Recent investigators have studied effects of carbohydrates on glycogen content, tissue size and shell size.

Haven (1965) showed that if oysters received wheatflour, cornstarch or dextrose in addition to their regular diet, they would, at certain seasons, have tissue weights significantly heavier than

controls receiving only flows of York River water. These studies were corroborated by Gillespie et al. (1966) who contended that oysters receiving cornmeal, wheatflour or dextrose had higher glycogen levels, more tissue and a higher rate of shell growth than controls. These authors, however, did not subject their data to statistical analysis, and their contention of positive results must, in many instances, be regarded as speculative.

The purpose of the present study was to add to the results of previous authors; that is, to investigate statistically effects of known quantities of supplements at various seasons on glycogen, tissue weight and shell size. The present study consisted of five experiments conducted from July 1966 to June 1967 at the Virginia Institute of Marine Science. Individual experiments investigated 1) the effect of various concentrations of cornstarch on glycogen content, tissue weight and shell size; 2) the effect of dextrose on glycogen content, tissue weight and shell size when given to oysters in two forms, dissolved and adsorbed on montmorillonite, a naturally occurring, inert clay; and 3) how effects of supplements may vary with the seasons.

METHODS AND MATERIALS

Oysters for all experiments were collected from Horsehead Bar in the James River, Virginia, an area free of known diseases which might influence tissue or shell growth (Andrews, personal communication). These were freed of attached fouling and held in a cold room prior to the start of each experiment. A day or two before the beginning of each study, oysters were removed from the cold room, selected for uniformity of size, randomly separated into groups of 20 and numbered with an enamel paint. After numbering, the following measurements were taken on the individual oysters: 1) underwater shell weight to the nearest 0.01 g by the method of Andrews (1961); 2) total weight in air to the nearest 0.01 g; 3) shell height to the nearest 0.1 mm; and 4) total volume to the nearest 0.1 cc by a water displacement method.

A group of 20 oysters was sacrificed on the first day of each study and the following additional measurements were taken: 1) shell volume to the nearest 0.1 cc by a water displacement method; 2) shell cavity volume to the nearest 0.1 cc was determined as the difference between total volume and shell volume; 3) wet tissue weight to the nearest 0.01 g; and 4) dry tissue weight to the nearest 0.01 g or glycogen content to the nearest 0.01% on a wet tissue weight basis. Wet tissue weight was recorded after the tissue had drained for one minute on a metal grid. Tissues analyzed for dry weight were placed

in an oven at 85 C for three days and then cooled in a desiccator prior to weighing.

Tissues analyzed for glycogen were placed in double plastic bags and stored in a freezer at -18 C. At a later date individual tissues were removed from the bags, diced, placed in 15 ml conical centrifuge tubes and homogenized with an ultra-sonic tissue disrupter (Branson Instruments Inc.). Two 50-150 mg samples were taken from each tissue homogenate and analyzed for glycogen. Glycogen was extracted by the method outlined by Calderwood and Armstrong (1941) as improved by Armstrong (unpublished). The quantity of glycogen was determined by the colorimetric method of Kemp and Kits van Heijningen (1954) and expressed as a per cent of the wet weight of the tissue sample. The two replications were averaged to determine the glycogen level for each oyster.

For each study the initial mean parameters of shell size outlined previously were compared by Student "t" tests at the 95% confidence level; significant differences between sacrificed and experimental groups were not shown in any study. Consequently, in any experiment it was assumed that groups had similar initial shell volumes, shell cavity volumes, tissue weights and glycogen levels. This assumption was necessary since it was impossible to make these measurements on live oysters.

Oysters were held in plexi-glass troughs under running York River water. Each trough measured 14" by 7 1/2" by 2 1/2" and consisted of five compartments for holding oysters and a baffle to insure thorough mixing of water and supplement (Fig. 1). Oysters were oriented in the troughs with bills facing into the current

Figure 1. Holding trough for oysters.

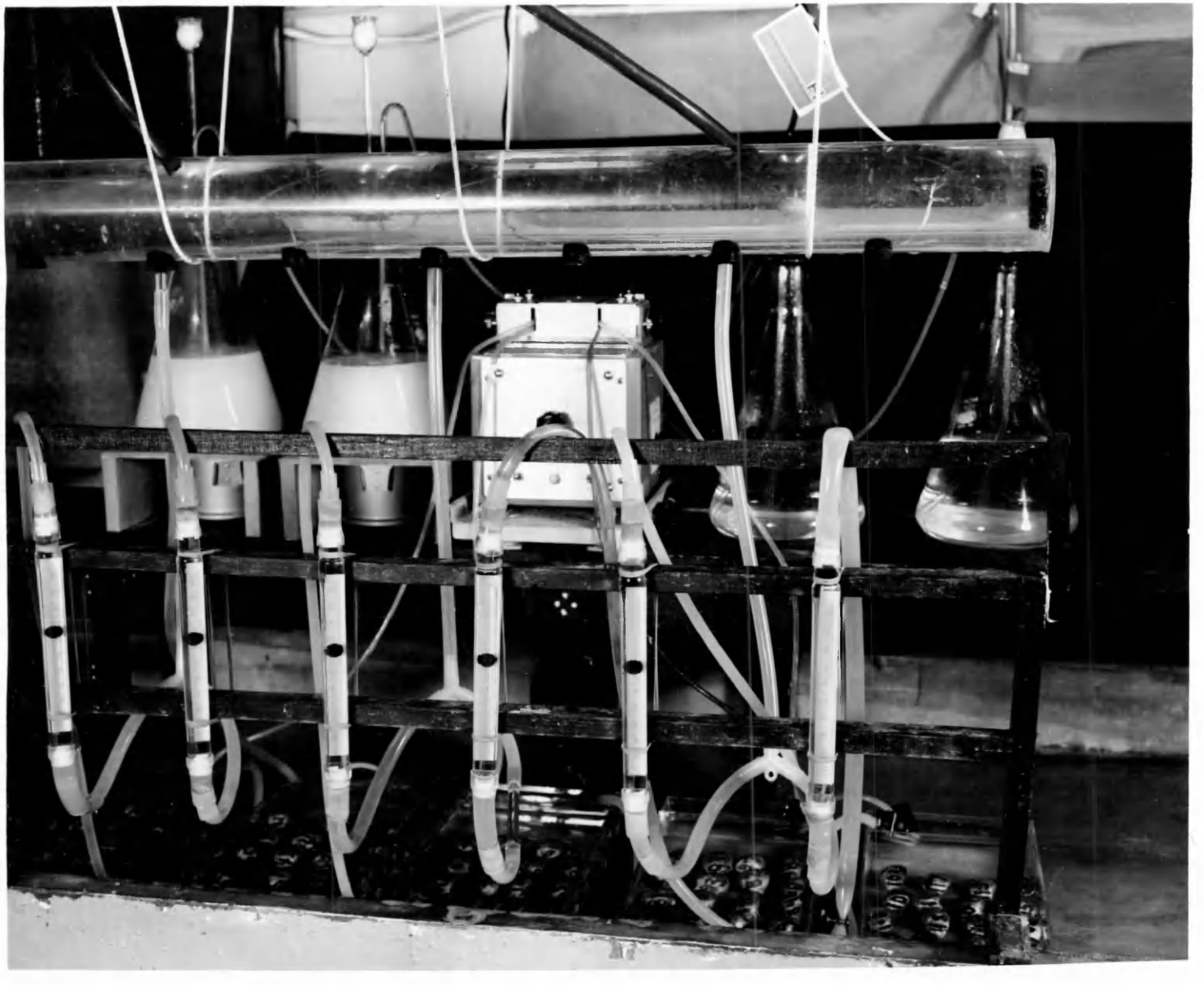
and the right valve up. Position of oysters in respect to the inflow was changed daily. Troughs were scrubbed and emptied of feces and pseudofeces every other day.

York River water, pumped to an overhead trough in the laboratory, flowed into a conducting column and through PVC tubing (Cole-Parmer Instrument Co.) to flowmeters (RGI Inc.) which monitored flows at a desired rate (Fig. 2). Water flow was checked twice a day and adjusted by screw clamps to the desired levels. Water temperature and salinity were not regulated but were the same as that of the York River. Temperatures were taken twice daily with a stem thermometer. Salinity determinations were made with a stem hydrometer.

Supplements were prepared by adding aliquots of starch, dextrose and montmorillonite to 3,000 ml of tapwater in 4,000 ml erlenmeyer flasks. The flasks were fitted with number 10, 2-holed rubber stoppers. One hole contained a short piece of glass tubing plugged with cotton and functioned as a vent; the second hole contained a glass tube which extended to the bottom of the flask and served as a delivery tube. Before use, supplements in their flasks, rubber stoppers and glass tubing were autoclaved for 10 minutes at 10 pounds pressure (115.5 C).

Supplements were delivered to the troughs through PVC tubing attached to the glass delivery tube. Flows were regulated by peristaltic pumps (Harvard Apparatus Co.) and checked twice a day. Particulate supplements were kept in suspension by means of magnetic bars and stirrers (Precision Scientific Co.). The content of each flask lasted about three days, being replaced by full, pre-sterilized, reserve flasks as they became empty.

Figure 2. Laboratory apparatus for delivery of water and supplements to control and test oysters.



At the end of each study, addition of supplements was stopped, and oysters received only York River water for one day to flush undigested supplement from their digestive tracts. Individual oysters were cleaned of attached fouling and measured for final underwater shell weight, total weight in air, shell height, total volume, shell volume, shell cavity volume, wet tissue weight and dry tissue weight or glycogen content.

Final analysis for differences which might have been produced by the various supplements was by Student "t" tests at the 95% confidence level. Analysis was divided into two phases: 1) final measurements were compared to initial measurements to determine the magnitude of increases in glycogen content, tissue weight and shell size in relation to controls (initial measurements of oysters which had died during a study were deleted and were not used in the comparison); and 2) final measurements of test groups were compared to final measurements of the control group to determine if significant differences occurred as a result of the addition of supplements. Linear regression analysis was used to determine if a correlation existed between mean glycogen content and wet tissue weight.

RESULTS

Experiment Ia

Experiment Ia, conducted in summer, extended 38 days from 7 July to 13 August 1966. During this time water temperatures were at a yearly maximum and averaged 25.6 C, with a range of 23.9-29.4 C. Salinity ranged from 20.4-22.4 o/oo and averaged 21.6 o/oo.

Six groups of oysters were used. Four served as test groups and were held in the laboratory in troughs. Three received starch, dextrose and montmorillonite separately at 2 mg/l each; the fourth received a mixture of 2 mg/l of dextrose and 2 mg/l of montmorillonite. A fifth group, also held in a trough in the laboratory, served as a control and received only York River water. Flow to each trough was 1 liter/min.

The sixth group was sacrificed on the first day of the experiment to determine initial glycogen content, wet tissue weight, shell volume and shell cavity volume.

Comparison between initial and final measurements. Oysters receiving dextrose, starch or the dextrose and montmorillonite mixture increased significantly in glycogen with final levels 2.3, 2.1 and 1.7 times greater than the initial level (Table 1). However, only the starch-fed group showed a significant increase in wet tissue weight. In respect to shell size, both groups receiving starch or the dextrose and montmorillonite mixture and the control

TABLE 1

Experiment Ia; 7 July-13 August 1966

Initial and final measurements on oysters

Group	Supplement	Concentration mg/l	Glycogen content % of wet tissue		Wet tissue weight g		Shell volume cc		Shell cavity volume cc	
			Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sacrificed	None		0.51	----	1.42	----	3.85	----	3.16	----
Control	None		----	0.47	----	1.50	----	5.31*	----	3.82*
Test	Starch	2	----	1.06*	----	1.75*	----	5.92*	----	3.83*
Test	Dextrose	2	----	1.17*	----	1.31	----	5.58*	----	3.60
Test	Montmorillonite	2	----	0.63	----	1.27	----	5.21*	----	3.74
Test	Dextrose & Montmorillonite	2 2	----	0.87*	----	1.40	----	5.69*	----	3.86*

*Final measurement is significantly different from initial, $P \leq 0.05$.

increased significantly over all initial measurements (Table 1,2). Oysters receiving dextrose or montmorillonite separately increased significantly in all shell parameters except shell cavity volume.

Comparison of final measurements between test and control groups. Glycogen levels were strongly influenced by starch, dextrose and the dextrose and montmorillonite mixture. Oysters receiving these supplements had final glycogen levels significantly higher than did controls (Table 3). In respect to wet tissue weight, significant differences between control and test groups did not occur. With a single exception, there was no significant difference between control and test groups in shell parameters. The group receiving montmorillonite had a final mean shell height significantly less than that of the control.

Experiment Ib

This study was carried out concurrently with experiment Ia, and laboratory conditions, supplements and concentrations, oyster groups and water flow were the same. It differed from Ia in that oyster tissue was analyzed for dry weight instead of glycogen content.

Comparison between initial and final measurements. Only oysters receiving starch showed a significant increase in dry and wet tissue weight (Table 4). Oysters receiving the dextrose and montmorillonite mixture showed a significant decrease in wet tissue weight. The remaining groups had final dry and wet tissue weights not significantly different from the initial weights. All groups receiving supplements

TABLE 2

Experiment Ia; 7 July-13 August 1966

Supplements, concentrations, final number of oysters and initial and final measurements on oysters

Group	Supplement	Concentration mg/l	Final no. of oysters	Underwater shell weight g		Total weight in air g		Shell height mm		Total volume cc	
				Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sacrificed	None		20	4.88	----	11.48	----	39.6	----	7.01	----
Control	None		19	5.33	7.21*	12.38	16.02*	42.4	45.5*	7.51	9.13*
Test	Starch	2	19	5.54	8.50*	12.16	17.87*	40.1	45.0*	7.08	9.75*
Test	Dextrose	2	20	5.45	7.81*	12.12	16.69*	38.9	44.0*	7.05	9.18*
Test	Montmorillonite	2	16	5.18	7.35*	11.77	15.97*	37.8	41.8*	7.11	8.96*
Test	Dextrose & Montmorillonite	2 2	20	5.46	7.75*	12.43	16.89*	41.5	46.1*	7.37	9.55*

*Final measurement is significantly different from initial, $P \leq 0.05$.

TABLE 3

Experiment Ia; 7 July-13 August 1966

Statistical comparison of final measurements between test and control groups

Test group	Glycogen content	Wet tissue weight	Shell weight	Total weight in air	Shell height	Total volume	Shell volume	Shell cavity volume
Starch 2 mg/l	+	0	0	0	0	0	0	0
Dextrose 2 mg/l		0	0	0	0	0	0	0
Montmo-rillonite 2 mg/l	0	0	0	0		0	0	0
Dextrose & Montmo-rillonite 2 mg/l each		0	0	0	0	0	0	0

+ = significantly greater than control, $P \leq 0.05$

0 = not significantly different from control, $P \leq 0.05$.

- = significantly smaller than control, $P \leq 0.05$.

TABLE 4

Experiment Ib; 7 July-13 August 1966

Initial and final measurements on oysters

Group	Supplement	Concentration mg/l	Dry tissue weight g		Wet tissue weight g		Shell volume cc		Shell cavity volume cc	
			Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sacrificed	None		0.31	----	1.71	----	4.62	----	3.97	----
Control	None		----	0.25	----	1.50	----	5.80*	----	4.53
Test	Starch	2	----	1.47*	----	2.23*	----	6.06*	----	4.58
Test	Dextrose	2	----	0.31	----	1.91	----	6.42*	----	4.91*
Test	Montmorillonite	2	----	0.31	----	1.80	----	6.02*	----	4.78*
Test	Dextrose & Montmorillonite	2 2	----	0.33	----	1.07*	----	6.70*	----	4.59

*Final measurement is significantly different from initial, $P \leq 0.05$.

increased significantly in shell weight, total weight, shell height, total volume and shell volume (Table 4,5). Controls increased significantly in all shell parameters except total volume. Only those groups receiving dextrose and montmorillonite separately increased significantly in shell cavity volume.

Comparison of final measurements between test and control groups. Only two supplements had an effect on tissue weight. Starch produced dry and wet tissue weights significantly heavier than those of the control (Table 6). The mixture of dextrose and montmorillonite produced wet tissue weight significantly less than that of the control. None of the supplements had a measurable effect on shell size.

Experiment II

This study lasted 47 days from 27 August to 11 October 1966, a period when water temperatures were decreasing. Water temperature averaged 22.1 C and ranged from 27.2-17.2 C. Salinity averaged 23.1 o/oo and ranged from 21.3-24.2 o/oo.

There were seven groups of oysters in this study. Six were held in troughs in the laboratory and treated as follows: one received starch at 2 mg/l; a second received starch at 5 mg/l; a third received dextrose at 2 mg/l; a fourth received montmorillonite at 2 mg/l; a fifth received a mixture of 2 mg/l of dextrose and 2 mg/l of montmorillonite; and the sixth served as a control and received only York River water. Flow to each laboratory group was 1 liter/min.

TABLE 5

Experiment Ib; 7 July-13 August 1966

Supplements, concentrations, final number of oysters and initial and final measurements on oysters

Group	Supplement	Concentration mg/l	Final no. of oysters	Underwater shell weight g		Total weight in air g		Shell height mm		Total volume cc	
				Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sacrificed	None		20	5.82	----	14.10	-----	44.2	----	8.59	----
Control	None		18	5.51	7.96*	13.41	17.82*	42.6	46.4*	8.37	10.33
Test	Starch	2	20	5.37	8.41*	12.82	18.66*	42.0	46.4*	8.02	10.64*
Test	Dextrose	2	18	6.09	9.06*	14.33	19.94*	43.0	47.4*	8.69	11.33*
Test	Montmorillonite	2	18	5.67	8.42*	13.57	18.78*	44.1	48.4*	8.39	10.80*
Test	Dextrose & Montmorillonite	2 2	15	6.56	9.48*	14.72	20.38*	42.3	46.2*	8.87	11.29*

*Final measurement is significantly different from initial, $P \leq 0.05$.

TABLE 6

Experiment Ib; 7 July-13 August 1966

Statistical comparison of final measurements between test and control groups

Test group	Dry tissue weight	Wet tissue weight	Shell weight	Total weight in air	Shell height	Total volume	Shell volume	Shell cavity volume
Starch 2 mg/l	+	+	0	0	0	0	0	0
Dextrose 2 mg/l	0	0	0	0	0	0	0	0
Montmo-rillonite 2 mg/l	0	0	0	0	0	0	0	0
Dextrose & Montmo-rillonite 2 mg/l each	0		0	0	0	0	0	0

+ = significantly greater than control, $P \leq 0.05$.

0 = not significantly different from control, $P \leq 0.05$.

- = significantly smaller than control, $P \leq 0.05$.

The seventh group was sacrificed on the first day to determine initial glycogen content, wet tissue weight, shell volume and shell cavity volume. Failure of the freezer in which this oyster tissue was stored prevented determination of the initial glycogen content.

Comparison between initial and final measurements. Increases over initial glycogen content could not be determined. All laboratory groups showed significant increases over initial wet tissue weight and shell parameters except shell cavity volume (Tables 7,8). Significant increases in shell cavity volume were limited to oysters receiving the starch supplements (2 and 5 mg/l).

Comparison of final measurements between test and control groups. All supplements produced final glycogen levels significantly higher than that of the control (Table 9). The highest levels were produced by the starch supplements. These levels were 13.6 (2 mg/l) and 15.6 (5 mg/l) times greater than the control level. Significantly heavier wet tissue weights and shell parameters were limited to the starch-fed oysters. Starch at both concentrations, however, had no demonstrable effect on shell height or shell cavity volume.

Experiment III

This study, carried out over a period of 48 days, extended from 21 October to 6 December 1966. During this time water temperature fell rapidly from a high of 18.2 C to a low of 5.6 C and averaged 12.6 C. Salinity ranged from 23.3-20.9 o/oo and averaged 22.0 o/oo.

Six groups of oysters were used. Four were held in the laboratory and treated as follows: one received starch at 2 mg/l;

TABLE 7

Experiment II; 27 August-11 October 1966

Initial and final measurements on oysters

Group	Supplement	Concentration mg/l	Glycogen content % of wet tissue		Wet tissue weight g		Shell volume cc		Shell cavity volume cc	
			Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sacrificed	None		----	----	1.37	----	4.39	----	3.77	----
Control	None		----	0.68	----	1.88*	----	6.21*	----	4.24
Test	Starch	2	----	9.26	----	2.92*	----	7.28*	----	4.68*
Test	Starch	5	----	10.59	----	3.24*	----	7.17*	----	4.82*
Test	Dextrose	2	----	1.62	----	1.95*	----	6.73*	----	4.28
Test	Montmorillonite	2	----	1.08	----	1.67*	----	6.45*	----	3.88
Test	Dextrose & Montmorillonite	2 2	----	1.23	----	2.00*	----	6.67*	----	4.35

*Final measurement is significantly different from initial, $P \leq 0.05$.

TABLE 8

Experiment II; 27 August-11 October 1966

Supplements, concentrations, final number of oysters and initial and final measurements on oysters

Group	Supplement	Concentration mg/l	Final no. of oysters	Underwater shell weight g		Total weight in air g		Shell height mm		Total volume cc	
				Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sacrificed	None		20	6.27	----	13.93	-----	40.9	----	8.15	-----
Control	None		20	6.08	8.40*	13.86	18.94*	41.5	47.8*	8.31	10.45*
Test	Starch	2	20	6.15	9.99*	13.89	22.08*	42.0	50.1*	8.19	11.96*
Test	Starch	5	20	6.28	9.70*	14.15	21.82*	41.2	49.2*	8.56	11.99*
Test	Dextrose	2	20	6.21	9.06*	13.75	20.14*	41.7	48.9*	8.08	11.01*
Test	Montmorillonite	2	20	6.48	8.83*	14.20	18.88*	41.8	46.1*	8.14	10.33*
Test	Dextrose & Montmorillonite	2 2	20	6.46	9.23*	14.28	20.57*	42.1	47.5*	8.31	11.02*

*Final measurement is significantly greater than initial, $P \leq 0.05$.

TABLE 9

Experiment II; 27 August-11 October 1966

Statistical comparison of final measurements between test and control groups

Test group	Glycogen content	Wet tissue weight	Shell weight	Total weight in air	Shell height	Total volume	Shell volume	Shell cavity volume
Starch 2 mg/l	+	+	+	+	0	+	+	0
Starch 2 mg/l					0			0
Dextrose 2 mg/l		0	0	0	0	0	0	0
Montmo-rillonite 2 mg/l		0	0	0	0	0	0	0
Dextrose & Montmo-rillonite 2 mg/l each		0	0	0	0	0	0	0

+ = significantly greater than control, $P \leq 0.05$.

0 = not significantly different from control, $P \leq 0.05$.

the second received starch at 5 mg/l; the third received dextrose at 2 mg/l; the fourth served as a control and received only York River water. Flow to each laboratory group was 1 liter/min. The fifth group was held in a wire tray suspended off the bottom of the York River. A sixth group was sacrificed at the start of the study to determine initial tissue and shell parameters.

Comparison between initial and final measurements. The increase in glycogen content of oysters held in the laboratory during this experiment was pronounced. Controls showed a 1.5-fold increase in glycogen over the initial level, dextrose-fed oysters showed a 2.5-fold increase, and oysters receiving starch at 2 and 5 mg/l showed a 5-fold and 6-fold increase, respectively (Table 10). Oysters held in the York River showed no measurable increase over the initial glycogen level.

In wet tissue weight, all laboratory groups had significant increases over the initial weight, with the starch supplements yielding the greatest increases. River oysters showed no significant increase over the initial wet tissue weight. Only oysters receiving starch at 5 mg/l increased significantly in all shell measurements (Tables 10,11). Oysters receiving starch at 2 mg/l and dextrose at 2 mg/l increased significantly in all initial shell measurements except shell height. Controls increased significantly in shell weight, total weight, shell volume and shell cavity volume, but not in shell height or total volume. River oysters showed no significant increase over any initial shell parameters.

TABLE 10

Experiment III; 21 October-6 December 1966

Initial and final measurements on oysters

Group	Supplement	Concentration mg/l	Glycogen content % of wet tissue		Wet tissue weight g		Shell volume cc		Shell cavity volume cc	
			Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sacrificed	None		1.84	----	1.86	----	5.19	----	3.81	----
River	None		----	2.08	----	1.80	----	5.61	----	4.21
Control	None		----	2.84*	----	2.31*	----	6.08*	----	4.47*
Test	Starch	2	----	9.13*	----	3.10*	----	6.15*	----	4.64*
Test	Starch	5	----	11.48*	----	3.09*	----	6.11*	----	4.79*
Test	Dextrose	2	----	4.64*	----	2.49*	----	5.86	----	4.57*

*Final measurement is significantly different from initial, $P \leq 0.05$.

TABLE 11

Experiment III; 21 October-6 December 1966

Supplements, concentrations, final number of oysters and initial and final measurements on oysters

Group	Supplement	Concentration mg/l	Final no. of oysters	Underwater shell weight g		Total weight in air g		Shell height mm		Total volume cc	
				Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sacrificed	None		20	6.73	----	15.24	-----	43.0	----	9.00	-----
River	None		19	6.57	7.28	15.74	16.67	45.5	45.0	9.48	8.92
Control	None		20	6.93	8.24*	16.03	18.39*	44.1	46.4	9.48	10.55
Test	Starch	2	20	6.92	8.43*	16.05	18.82*	44.2	46.6	9.55	10.79*
Test	Starch	5	20	6.87	8.32*	16.32	18.86*	44.9	47.3*	9.84	10.90*
Test	Dextrose	2	20	6.81	8.13*	15.69	18.06*	44.5	46.3	9.29	10.43*

*Final measurement is significantly different from initial, $P \leq 0.05$.

Comparison of final measurements between test and control groups. All supplements yielded final glycogen levels significantly higher than control (Table 12). Starch at both concentrations produced the highest levels, these being 3.2 (2 mg/l) and 4.0 (5 mg/l) times greater than the control level. Oysters held in the York River had 27% less glycogen than controls, this difference being significant.

Only the starch supplements produced wet tissue weights significantly heavier than in the controls. River oysters had wet tissue weights significantly less than the controls by 22%. Supplements had no significant influence on shell parameters. River oysters were significantly smaller than controls in shell weight but not statistically different from the controls in the other shell parameters.

Experiment IV

This study lasted 50 days and extended from 1 May to 19 June 1967. Water temperature increased during this study from 12.8 C to 25.6 C and averaged 18.2 C. Salinity averaged 19.1 o/oo and ranged from 20.7-17.9 o/oo. Nine groups of oysters were used. Seven were held in the laboratory, one was held in the York River, and the ninth was sacrificed on the first day of the study to determine initial tissue and shell parameters.

In the laboratory, one group served as a control and received only York River water. Two served as starved groups and received York River water which had been pre-filtered by 50 oysters; one received no supplement, the other received starch at 2 mg/l. Two groups received starch at concentrations of 0.25 and 0.5 mg/l and

TABLE 12

Experiment III; 21 October-6 December 1966

Statistical comparison of final measurements between test and control groups

Test group	Glycogen content	Wet tissue weight	Shell weight	Total weight in air	Shell height	Total volume	Shell volume	Shell cavity volume
River		-		0	0	0	0	0
Starch 2 mg/l		+	0	0	0	0	0	0
Starch 5 mg/l			0	0	0	0	0	0
Dextrose 2 mg/l		0	0	0	0	0	0	0

+ = significantly greater than control, $P \leq 0.05$.

0 = not significantly different from control, $P \leq 0.05$.

- = significantly smaller than control, $P \leq 0.05$.

normal water. The sixth group received dextrose at 5 mg/l and normal water, and the seventh group received a mixture of 5 mg/l of dextrose and 2 mg/l of montmorillonite and normal water. Water flow, less than in all previous experiments, was only 1/2 liter/min.

Comparison between initial and final measurements. Oysters receiving supplements, river oysters and controls increased significantly in glycogen content during this study (Table 13). The starved oysters which did not receive a starch supplement showed a significant decrease of 48% in glycogen from the initial level. Starved oysters receiving starch at 2 mg/l showed a significant, 6.5-fold increase in glycogen over the initial level. Significant increases over initial wet tissue weight were demonstrated by all groups except the starved groups.

Significant increases over initial shell measurements were realized by test, control and river groups in shell weight, total weight, shell height, total volume and shell volume (Tables 13,14). Only oysters receiving starch at 0.5 mg/l had a significant increase over initial shell cavity volume. The starved oysters receiving starch at 2 mg/l increased significantly over all initial shell measurements except total volume and shell cavity volume. Starved oysters receiving only filtered water showed a significant decrease in shell cavity volume.

Comparison of final measurements between test and control groups. Five groups had final glycogen levels significantly different from the control level (Table 15). Starch at 0.25 and 0.5 mg/l produced significantly more glycogen than that of the control. River oysters and starved oysters not receiving the starch supplement had

TABLE 13

Experiment IV; 1 May-19 June 1967

Initial and final measurements on oysters

Group	Supplement	Concentration mg/l	Glycogen content % of wet tissue		Wet tissue weight g		Shell volume cc		Shell cavity volume cc	
			Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sacrificed	None		1.67	----	1.91	----	5.27	----	4.57	----
River	None		----	2.33*	----	2.94*	----	7.52*	----	4.72
Control	None		----	3.08*	----	2.46*	----	6.99*	----	4.33
Starved	None		----	0.87*	----	2.01	----	5.72	----	3.82*
Starved	Starch	2	----	10.82*	----	2.20	----	6.19*	----	4.02
Test	Starch	0.25	----	4.58*	----	2.33*	----	6.99*	----	4.61
Test	Starch	0.5	----	4.73*	----	2.82*	----	6.96*	----	5.37*
Test	Dextrose	5	----	3.48*	----	2.69*	----	7.21*	----	4.88
Test	Dextrose & Montmorillonite	5 2	----	3.02*	----	2.49*	----	7.19*	----	4.83

*Final measurement is significantly different from initial, $P \leq 0.05$.

TABLE 14

Experiment IV; 1 May-19 June 1967

Supplements, concentrations, final number of oysters and initial and final measurements on oysters

Group	Supplement	Concentration mg/l	Final no. of oysters	Underwater shell weight g		Total weight in air g		Shell height mm		Total volume cc	
				Initial	Final	Initial	Final	Initial	Final	Initial	Final
Sacrificed	None		20	6.88	----	16.33	-----	46.1	----	9.84	-----
River	None		20	6.82	10.30*	16.14	22.08*	44.4	47.8*	9.76	12.24*
Control	None		19	6.98	9.47*	16.00	20.57*	44.2	49.3*	9.44	11.32*
Starved	None		19	7.03	7.36	16.14	16.67	44.1	44.4	9.54	9.54
Starved	Starch	2	20	7.03	8.53*	16.00	18.50*	43.8	47.4*	9.32	10.21
Test	Starch	0.25	20	6.98	9.59*	15.91	20.82*	43.8	50.6*	9.30	11.60*
Test	Starch	0.5	20	6.68	9.47*	16.17	21.36*	45.8	52.3*	9.86	12.33*
Test	Dextrose	5	20	6.96	9.91*	16.22	21.55*	45.2	50.9*	9.52	12.09*
Test	Dextrose & Montmorillonite	5 2	20	7.08	10.10*	16.10	21.73*	44.8	50.8*	9.45	12.02*

*Final measurement is significantly different from initial, $P \leq 0.05$.

TABLE 15

Experiment IV; 1 May-19 June 1967

Statistical comparison of final measurements between test and control groups

Test group	Glycogen content	Wet tissue weight	Shell weight	Total weight in air	Shell height	Total volume	Shell volume	Shell cavity volume
River		+	0	0	0	0	0	0
Starved								0
Starved (Starch 2 mg/l)		0			0			0
Starch 0.25 mg/l		0	0	0	0	0	0	0
Starch 0.5 mg/l			0	0		0	0	
Dextrose 5 mg/l	0	0	0	0	0	0	0	0
Dextrose & Montmorillonite 5 & 2 mg/l	0	0	0	0	0	0	0	0

+ = significantly greater than control, $P \leq 0.05$.
0 = not significantly different from control, $P \leq 0.05$.
- = significantly smaller than control, $P \leq 0.05$.

significantly less glycogen than controls. Starved oysters receiving starch at 2 mg/l had 3.5 times more glycogen than controls and 12.4 times more glycogen than starved oysters not receiving the starch supplement. Dextrose and the dextrose and montmorillonite mixture had no significant effect on final glycogen levels.

Oysters receiving starch at 0.5 mg/l and the river oysters had wet tissue weights significantly heavier than that of controls, but starch at 0.25 mg/l had no significant effect. Starved oysters not receiving the starch supplement had wet tissue weights significantly smaller than that of controls. Starved oysters receiving starch and the groups receiving dextrose and the dextrose and montmorillonite mixture had wet tissue weights not significantly different from that of controls.

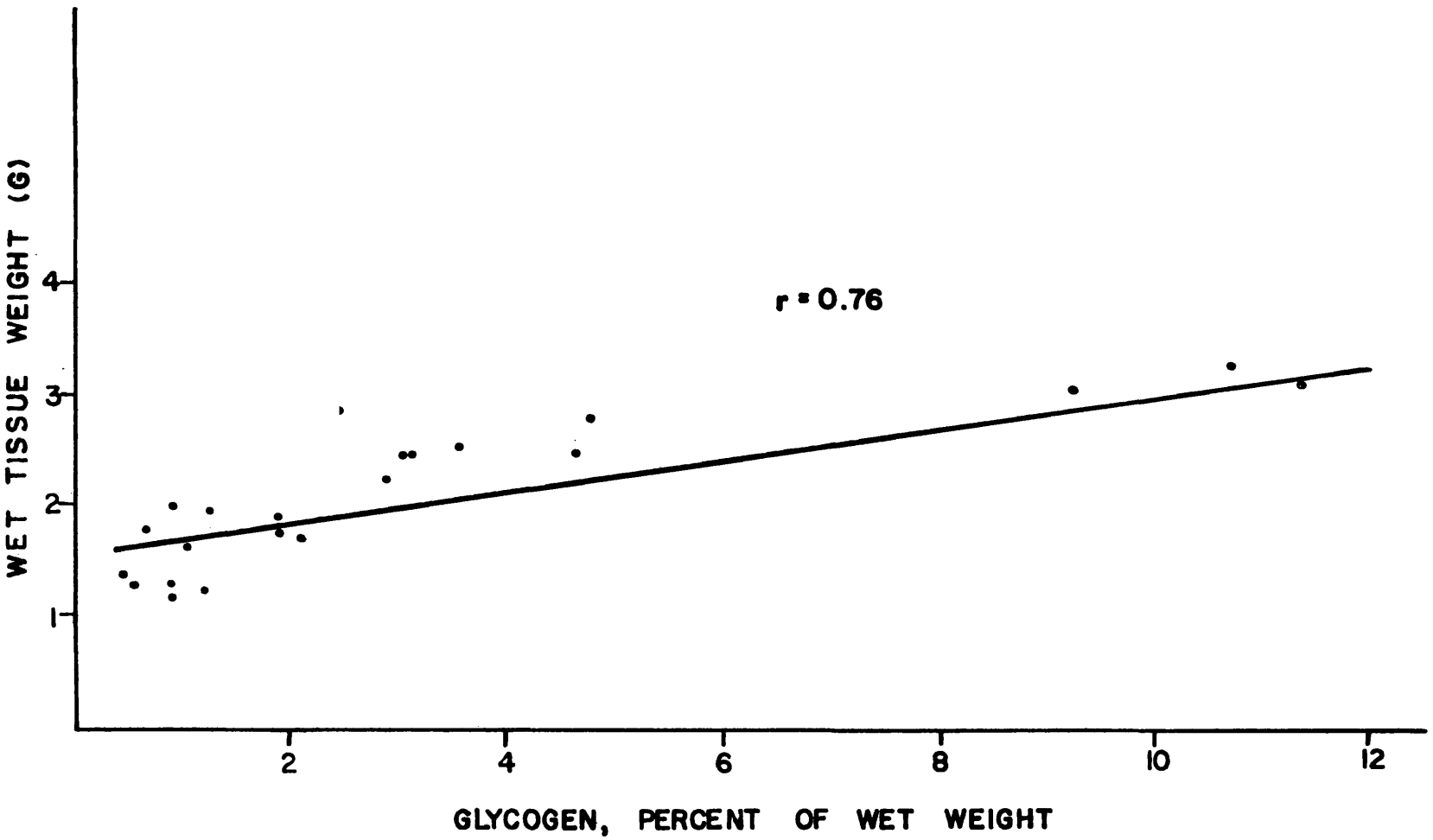
Significant differences between the control and other groups occurred in shell measurements. Starch at 0.5 mg/l produced shell heights and shell cavity volumes significantly greater than those of controls. Both starved groups had shell weights, total weights, total volumes and shell volumes significantly less than those of the controls. In addition, starved oysters not receiving the starch supplement were significantly smaller than the starved oysters receiving starch in shell weight, total weight and shell height. Starch at 0.25 mg/l, dextrose and the dextrose and montmorillonite mixture had no significant effect on shell parameters.

Relation of glycogen to wet tissue weight

An analysis of the mean data of all experiments conducted during summer, fall and spring showed a positive correlation of 0.76

between glycogen content and wet tissue. The linear regression of the data in Tables 1, 4, 7, 10 and 13 is shown in Figure 3: wet tissue weight = 1.59 + 0.13 glycogen content.

Figure 3. Linear regression; glycogen versus tissue weight.


WET TISSUE WEIGHT (G)
1
2
3
4
r = 0.76
2
4
6
8
10
12
GLYCOGEN, PERCENT OF WET WEIGHT

DISCUSSION

Comparison of initial measurements to final measurements is a necessary aspect of this study. Comparison of final measurements only would not have presented a complete concept of effects of supplemental feeding since significant differences might have been due to decreases in control measurements. If adverse conditions had existed in the laboratory, the addition of supplements might have partially or completely compensated for them. The controls, however, would have been subject to the full extent of these adverse conditions.

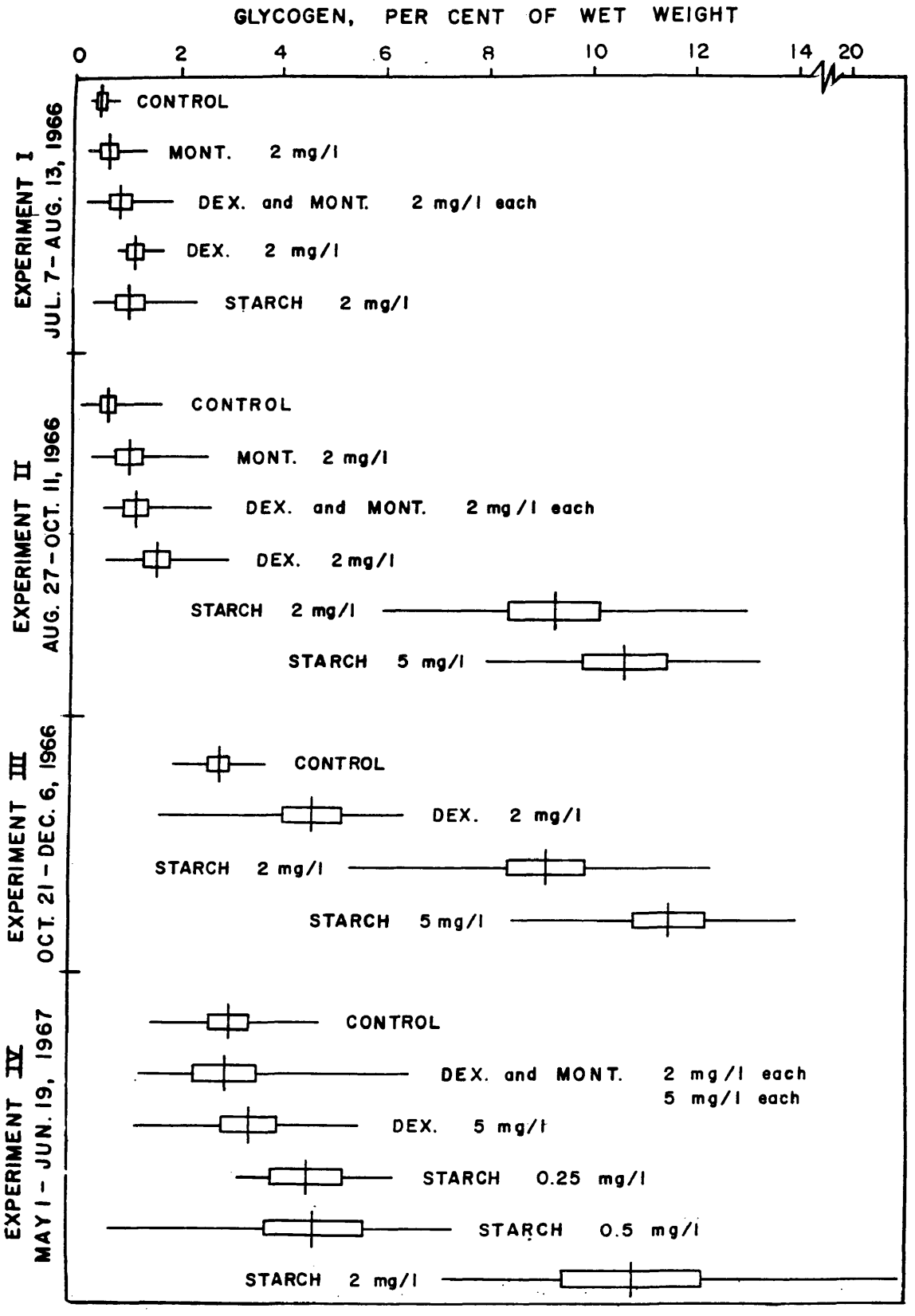
This comparison showed that control oysters held in the laboratory increased in glycogen content, tissue weight and shell size during all experiments except those conducted in the summer. In experiments III and IV these increases equaled or exceeded those of oysters held in the York River (river oysters were used in these two experiments only). This clearly indicates that significant differences in final measurements for all studies were due to the addition of supplements and not adverse laboratory conditions which would inhibit increases or bring about decreases in the glycogen content, tissue weight or shell size of controls. Although controls did not increase in tissue weight or glycogen during the summer, it is felt that this was due to their physiological state at this time and not to adverse laboratory conditions. The remainder

of this discussion will be devoted to the comparison of final measurements between test and control groups.

Control oysters in individual experiments showed significant differences in glycogen with low levels in summer and early fall and high levels in late fall and spring (Fig. 4). This agrees with the ecology of the oyster since fall and spring are periods of glycogen storage, the maximum levels being attained in the latter part of each season. These seasonal changes are typical of oysters growing in the York River and other localities (Galtsoff et al., 1947). Statistical analysis in each of the five studies showed that effects of supplements on glycogen content varied with season and concentration (Fig. 4). Consequently, effects of supplements must be considered in relation to the basic glycogen cycle.

Of the supplements tested, cornstarch had the greatest effect on glycogen content, tissue weight and shell size. During the warmer summer period (Experiments Ia and Ib) minimal results were obtained with starch at 2 mg/l. However, it is pertinent that even at this time starch had a significant effect on glycogen content and dry tissue weight. This positive influence of starch resulted in 2.3 times more glycogen and 1.5 times heavier dry tissue than that in the control. It is postulated that the comparatively low glycogen levels obtained during the summer were due to the fact that, if glycogen were being formed, it was being utilized in the formation of eggs and sperm. The lack of starch influence on shell size and wet tissue weight at this time may be associated with the absence of stored glycogen.

Figure 4. Seasonal variation in glycogen of control and test oysters; range, mean and interval estimate ($\pm t.05S_{\bar{x}}$).


GLYCOGEN, PER CENT OF WET WEIGHT
0
2
4
6
8
10
12
14
20
EXPERIMENT I
JUL. 7 – AUG. 13, 1966
CONTROL
MONT. 2 mg/l
DEX. and MONT. 2 mg/l each
DEX. 2 mg/l
STARCH 2 mg/l
EXPERIMENT II
AUG. 27 – OCT. 11, 1966
CONTROL
MONT. 2 mg/l
DEX. and MONT. 2 mg/l each
DEX. 2 mg/l
STARCH 2 mg/l
STARCH 5 mg/l
EXPERIMENT III
OCT. 21 – DEC. 6, 1966
CONTROL
DEX. 2 mg/l
STARCH 2 mg/l
STARCH 5 mg/l
EXPERIMENT IV
MAY 1 – JUN. 19, 1967
CONTROL
DEX. and MONT. 2 mg/l each
5 mg/l each
DEX. 5 mg/l
STARCH 0.25 mg/l
STARCH 0.5 mg/l
STARCH 2 mg/l

Maximum response to starch and other supplements in respect to glycogen occurred in the early and late fall (Experiments II and III). During each of these two studies similar starch concentrations were used (2 and 5 mg/l). In each experiment 5 mg/l produced significantly more glycogen than 2 mg/l, but levels produced by similar concentrations in early and late fall were not significantly different.

The magnitude of difference between starch-fed and control oysters varied between early and late fall. This was due to the fact that the final glycogen level of the control in late fall was four times that of the control in the early fall (Fig. 4). This resulted in starch producing 13.6 (2 mg/l) and 15.6 (5 mg/l) times more glycogen than the control level in early fall and only 3.2 (2 mg/l) and 4.0 (5 mg/l) times more in the late fall.

The high glycogen levels produced by starch supplements in both fall studies were associated with significantly heavier wet tissue weights than the controls. Starch, however, had a significant effect on shell size in early fall but had no effect in late fall. Interpretation of this data is complex and will be presented later.

At the termination of the late spring study (Experiment IV), the glycogen level of the control was high and was similar to that of the control in late fall. This again agrees with known aspects of the glycogen cycle in oysters; that is, during spring there is an accumulation of glycogen prior to gonadal development. At this time starch at 0.25 and 0.5 mg/l had a significant influence on glycogen levels. These low concentrations yielded final glycogen levels one-half of those produced by the 2 and 5 mg/l concentrations during early and late fall. This point is emphasized since it shows that

during spring starch concentrations only one-tenth of those used during the preceding fall produced one-half of the glycogen yielded by the higher concentrations. It should also be emphasized that during the spring experiment water flow was only one-half that used in the preceding studies. It would have been of interest to use starch at 2 and 5 mg/l in the late spring since the glycogen levels produced by these higher concentrations might have been greater than those yielded in the fall.

Starch at 0.5 mg/l, in addition to influencing glycogen content, also had a significant, positive effect on wet tissue weight, shell height and shell cavity volume, but the 0.25 mg/l concentration had a significant influence only on glycogen. This suggests that starch at 0.5 mg/l approaches the lower level which will influence tissue and shell size.

The effect of starch on shell size is difficult to interpret. Significant effects of starch on glycogen and tissue weight at all seasons (summer, fall and spring) were not always accompanied by significant influence on shell size. Minimal glycogen levels and tissue weights produced by starch in summer were not accompanied by increased shell growth. Final glycogen levels produced by starch at 2 and 5 mg/l in early fall and late fall were similar. However, positive effects of starch on shell size occurred only during early fall. In late spring effects of starch at 0.5 mg/l on glycogen and tissue weight were associated with effects on shell height and shell cavity volume. These results indicate that effects of starch on shell size may be limited to early fall and late spring.

Oysters receiving dextrose had higher glycogen levels than controls in summer and early and late fall. Effects of dextrose, however, were limited to an increase in glycogen; a significant influence on tissue weight or shell size was never demonstrated in any of the five studies. The influence of dextrose on glycogen levels was generally much less than that of starch. In early fall dextrose yielded only one-sixth of the glycogen produced by starch at the same concentration. In late spring starch at the trace quantities of 0.25 and 0.5 mg/l produced significantly more glycogen than did dextrose at 5 mg/l.

Results with dextrose in this study agree with those of previous authors. Haven (1965) showed that dextrose at 5 mg/l had no significant effect on tissue weight but did at the high concentration of 34 mg/l. Gillespie et al. (1966) also concluded that dextrose was limited in value as a supplement for oysters.

The use of montmorillonite as an adsorption media for dextrose was based on work by Bader (1962) who showed adsorption of organic sugars on clay particles. Although the mixture of dextrose and montmorillonite had a significant effect on glycogen levels in summer and early fall, it yielded significantly less glycogen than did dextrose alone. It is possible that the oysters were not capable of stripping off the dextrose absorbed on the clay particles. Montmorillonite was used as a control for the dextrose and montmorillonite mixture since clay had no nutritional value. Consequently, it was surprising that, in the early fall, montmorillonite alone had a positive influence on glycogen content. The reason for this is unknown.

Up to the present study no statistical relationship between glycogen content and tissue weight or volume had been demonstrated. Results of Hopkins et al. (1953) indicated that tissue volume followed the same seasonal cycle as glycogen, but statistical analysis was not employed. Gillespie et al. (1966) indicated that a relationship between glycogen content and tissue weight exists but did not analyze these results statistically. The present study has shown that a definite correlation between glycogen and wet tissue weight exists ($r = 0.76$). Thus, increased glycogen levels produced by supplements would have a definite effect on tissue size. This would yield more "meat" per bushel on a commercial basis. This relation supports the statement of Mitchell (1917) that investigations leading to increased meat yields must consider supplements which influence glycogen formation.

SUMMARY

Oysters were held in the laboratory and received natural York River water supplemented with low concentrations of cornstarch, dextrose, montmorillonite or a mixture of dextrose and montmorillonite. Effects of supplements on glycogen content, tissue weight and shell size in the summer, fall and spring were tested statistically.

Of the supplements used, starch had the most influence on glycogen content, tissue weight and shell size. Effect of starch varied with season, the maximum influence occurring in the early fall when water temperature was decreasing and spawning had ended, and the minimum influence occurring in summer when water temperature was at its yearly maximum and oysters were spawning. Effects of starch on glycogen content and tissue weight in the late fall were similar to those in the early fall. However, in the late fall starch had no influence on shell size. Influence of starch on oysters appeared intermediate in late spring. Starch at 0.5 mg/l had a definite influence on glycogen content, tissue weight and shell size. This low concentration appears to be the minimum which will influence tissue weight and shell size.

Results strongly suggest that dextrose, at the concentrations tested (2 and 5 mg/l), is of low supplemental value. Absorption of dextrose on montmorillonite decreased its supplemental value.

The relationship between glycogen content (G) and wet tissue weight (W) can be expressed by the regression equation $W = 1.59 + 0.13G$, $r = 0.76$. This indicates that glycogen content has a definite influence on tissue size and quality.

APPENDIX

Procedure for the Determination of Glycogen in Oysters

1. Dice oyster tissue in a watch glass.
2. Pour diced tissue into a 15 ml centrifuge tube and homogenize for two minutes with an ultra-sonic tissue disrupter (during disruption the tube should be suspended in an ice water bath).
3. Add 50-150 mg of homogenized tissue to a 15 ml centrifuge tube (do two replications for each oyster).
4. Add 1 ml of 30% NaOH (w/w) to the tissue sample and digest for 30 minutes in a boiling water bath.
5. Dilute the digested sample with 5-6 ml of hot distilled water from a wash bottle or a burette (if some of the tissue is still in suspension, slight stirring with a clean glass rod will dissolve it).
6. Add 7 ml 95% ethanol from a wash bottle with pressure to insure complete mixing, cap and let stand for 12 hours at room temperature.
7. Remove cap and centrifuge for 5 minutes, discard the supernatant, rinse the precipitated glycogen twice with 2 ml of 66% ethanol and discard the washings.
8. Dissolve the glycogen in 10 ml of hot distilled water.
9. If necessary, add 1 ml of the glycogen solution to 9 ml of distilled water for a final dilution factor of 100 (the added

dilution may be necessary if the amount of glycogen in the sample is high).

10. To 3 ml of concentrated H_2SO_4 (96%; sp. gr. = 1.84) add 1 ml of the diluted glycogen solution, mix thoroughly and heat for 6.5 minutes in a boiling water bath (during the color reaction the tubes should be partially capped; I have found that heat-resistant polypropylene caps serve this function well).
11. Cool the tubes under running tapwater to room temperature and read the adsorbance at 520 mu in a colorimeter or spectrophotometer.

NOTES:

A. Prepare a reagent blank by mixing 1 ml of distilled water with 3 ml of H_2SO_4 and treat the same as the glycogen-H_2SO_4 solution in step 10.

B. H_2SO_4 has the same adsorbance as water at 520 mu, so a distilled water blank is not necessary.

C. Prepare a stock solution of glucose by dissolving 1 g of glucose in distilled water and bring the volume up to 1 liter. A working solution is prepared by adding 1 ml of the stock solution to 9 ml of distilled water for a final glucose concentration of 0.1 mg/ml.

D. A standard solution of 0.1 mg of glucose/ml of distilled water has an adsorbance value of around .150-.160 (the actual value is dependent on the type and purity of H_2SO_4 used).

E. Formula used for determining the amount of glycogen in the sample:

$$\frac{\frac{\text{A. of sm.} \times 0.9}{\text{A. of st.}} \times 0.1 \text{ mg} \times \text{dil. fac.}}{\text{Weight of tissue sample in mg}} \times 100 = \% \text{ glycogen in sample}$$

0.1 mg = concentration of glucose in mg in standard solution.

0.9 = standard value for converting glycogen to glucose units.

sm. = sample.

st. = standard.

dil. fac. = dilution factor.

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VITA

Kenneth Wayne Turgeon

Born in Acushnet, Massachusetts, 10 February 1943. Graduated from Dartmouth High School, North Dartmouth, Massachusetts, June 1960. Attended Boston University, College of Basic Studies, Boston, Massachusetts, September 1960-June 1961. B.S. in Biology, Stonehill College, North Easton, Massachusetts, June 1965.

Entered the School of Marine Science of The College of William and Mary in Virginia, September 1965. Received a graduate assistantship in the Applied Science Department, March 1966. Taught biology and earth science, Gloucester High School, Gloucester County, Virginia, January 1968-June 1968.